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"Research in Photosynthesis"

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RESEARCH IN PHOTOSYNTHESIS

During the last reporting period we have continued our earlier studies in several areas of photosynthesis. These areas concerned quantum yields in chloroplast reactions as a function of wavelength, analysis of the oxygen evolving photoreaction by means of fluorescence and difference spectroscopy as well as studies of the role of manganese in photosynthesis. Drs. Kok and Cheniae presented papers at a recent A.E.C. sponsored meeting on photosynthesis in Brookhaven. A copy of Dr. Cheniae's paper which contains a brief survey of his manganese work up to date is included. A copy of Dr. Kok's paper which surveys the fluorescence and other kinetic studies is presently being prepared and will be forwarded upon completion.

STUDIES ON FUNCTION OF MANGANESE IN PHOTOSYNTHESIS [1]

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INTRODUCTION

For some time, since investigations of Pirson[1], Kessler et al[2,3,5] and Eyster et al[4], the implication has been in the literature that manganese is in some way involved in photosynthesis or the Hill reaction. In most instances, effects of manganese deficiency on these reactions observed by past workers have been made with tissues relatively aged or in many cases of uncertain age. Thus, it could be considered possible that effects of Mn deficiency observed were secondary rather than primary effects. To date there are no completely convincing data to indicate that manganese directly participates in a primary reaction of either photosynthesis or the Hill-reaction. Nevertheless many schemes of photosynthesis dogmatically include manganese as a catalyst on the oxidant side of photosystem II. Only within the past few years has there been renewed interest in the direct participation, if any, of manganese in photosynthesis. The data presented briefly represent some of our attempts to evaluate critically the function of manganese in photosynthesis, and to examine the manner in which manganese is bound within the chloroplast.

METHODS

The alga Scenedesmus D_3 has been used throughout these studies for both whole cell studies and as a source of chloroplasts. Manganese deficiencies resulting in ~ 90% loss of growth or photosynthesis can be obtained within 48 hours when "Specpure" chemicals are used as source of

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micro-elements. Unless otherwise stated quantum yield measurements (relative and absolute) were made employing an O_2-electrode. Absorption measurements were made with an Ulbricht integrating sphere. Radioactivity measurements of ^{54}Mn were made with a NaI scintillation well-counter calibrated to isolate the ^{54}Mn γ-emission.

RESULTS

A. Effect of Mn Deficiency on Photosynthesis, Whole Cell and Chloroplast Hill Reaction and Photosystem I Reactions.

Deficiency of manganese results in effects dependent upon the level of deficiency. At low levels of deficiency the effect observed upon either photosynthesis or the quinone Hill reaction is upon K_1, the rate constant for the limiting light-reaction[8] (Fig. 1). With a high level of deficiency, but yet from 48 hour cells, two effects are observed both in whole cells (Fig. 2) and in particles derived from the same cells (Fig. 3). Manifestation of the effects of deficiency is now seen both on K_1 and K_d in the same order of magnitude for Hill-reaction in particles as in whole cells. The primary effect of extreme deficiency observed by this type kinetic analysis is, however, on the K_1 rate constant. Flashing-light kinetic analyses are needed to completely resolve the dilemma of whether or not the deficiency directly effects a light reaction. The fact that the absolute quantum yield per O_2 of 16-20 quanta (650 mμ-680 mμ) for Hill-reaction in normal particles gives us some assurance that particle preparation is not creating artifacts that seriously jeopardize a kinetic analysis. In the Hill-reaction, the same results are obtained whether one uses high potential acceptors such as FeCN, cytochrome c or quinone or low potential acceptors such as

viologen or NADP. Such an observation lends support to the concept that the effect of Mn-deficiency is primarily upon photosystem II[7,9].

This concept is further supported by data of relative quantum yield measurements with Mn-deficient or normal particles on reactions sensitized by photosystem I. With particles showing essentially no Hill-activity, the photo-oxidation of reduced cytochrome c or the photoreduction of NADP mediated by ascorbate-DPIP shows identical quantum yields as compared to particles from non-deficient Scenedesmus (Fig. 4).

It was considered, however, that such experiments did not preclude the possibility that within photosystem I a site of manganese function existed with an affinity constant many fold greater than that within photosystem II. Since it is impossible to culture cells autotrophically in complete absence of manganese, we sought another means to eliminate all possibility of a function of manganese within photosystem I.

Chloroplast particles obtained from ^{54}Mn-uniformly labeled cells bind the incorporated manganese tenaciously. By several procedures, however, the simplest of which is a 50° heat treatment it is possible to remove all manganese from the particle without loss of photosystem I activity. There seems to be no direct correlation between loss of Mn and loss of Hill activity as judged by the dissimilarity in their kinetics (Fig. 5). It is clear, however, that photosystem I activity (photo-oxidation of reduced cytochrome c[10] or $DPIPH_2$ photoreduction of NADP)[11] is not influenced either in quantum yield or saturation rate by complete removal of chloroplast-bound manganese (Fig. 4).

The effects of manganese deficiencies on photosynthesis or the Hill-reaction are observed without a decrease in total chlorophyll b or carotenoids. In fact, in agreement with reports of Spencer and Possingham,[6] we consistently find in extremely Mn-deficient particles a small but significantly greater amount of chlorophyll b and possibly carotenoids than in the normal particles (Fig. 6). Conditions of deficiency affecting only the rate-limiting light reaction result in no change in the chl a/chl b ratio. These results indicate therefore that the effect of deficiency most probably is not a gross alteration of pigment composition.

Low temperature (-196°) fluorescence emission[12,13] analyses do however, reveal striking differences between the deficient and normal particles (Fig. 7). Both the F_{685} and F_{698} bands are diminished, whereas the F_{730} is increased in comparison to these emissions in normal particles. Upon restoration of Hill-activity in deficient algae by addition of manganese the relative emissions of F_{685}, F_{698}, and F_{730} restore to their "normal" ratios. More data are needed, however, to establish clearly that these effects on fluorescence emission are specifically and directly related to manganese and not simply a result of differences of particle size.

B. Restoration of Hill-Activity in Deficient Cultures by Light and Mn^{2+} in Absence of Protein and Net Chlorophyll Synthesis.

Many workers in the past have observed restoration of photosynthesis or Hill-reaction activity in Mn-deficient algae by addition of manganese salts to whole cells. Such experiments do not indicate whether the restorative effect is direct by binding of manganese to an "apoenzyme" or indirect

by an increase in general cellular material syntheses dependent upon manganese.

To delineate some of these possibilities, restoration experiments were made under conditions where protein synthesis was severely limited (~ 90%) by cycloheximide (actidione).[14,16] The results obtained were independent of time (15 minutes to ~ 4 hours) needed for complete restoration. In addition to inhibiting the incorporation of ^{14}C-phenylalanine into TCA-precipitable protein, this inhibitor also effectively blocks net chlorophyll synthesis[17] (Fig. 8). Despite these limitations upon the cell, no inhibitory effect on restoration of the Hill-reaction is observed (Fig. 9). These observations lend support to the hypothesis of a direct function of manganese in photosynthesis. Until, however, in vitro restoration of Hill-activity can be shown, the question remains unresolved.

The requirement of light for restoration (Fig. 9), which under the experimental conditions employed is always observed, is in conflict with the report of Arnon.[18] This light requirement has not been investigated in detail but exposure time seems to be of more importance than intensity. The light requirement cannot be explained simply as causing a large increase of flux of Mn^{2+} ions into the cell. It could possibly be related to a) oxidation of Mn^{2+} to an "active" oxidation-state or b) a configurational change of an Mn-apoenzyme enabling it to bind its manganese coenzyme. At this time no data are available to exclude these or other speculative hypotheses.

C. Nature of Chloroplast Bound Manganese.

The literature contains speculation concerning the manner in which manganese is bound within the chloroplasts. Suggestions have been made that manganese is bound within a "special chlorophyll" or a "special cytochrome." To date, however, there is little information to assess these hypotheses, nor is there agreement in the literature concerning the amount of manganese per unit of chlorophyll. If indeed, manganese is a functional component within photosystem II, additional data are needed on 1) nature of binding, 2) amount; and 3) the valency state(s) of the metal ion. Using the ^{54}Mn-chloroplasts from uniformly labeled Scenedesmus, attempts have been made to answer some of these questions.

The data of Table I represent the distribution of ^{54}Mn within fractions of particles that had been dialyzed overnight against de-ionized water. Extractions at -15° (to minimize degradation of a possible labile Mn-chlorophyll) with solvents to remove lipid revealed only a very small percentage of the total radioactivity associated with lipid-chlorophyll fraction.[19] Partitioning of the lipid phase with water resulted in complete loss of all ^{54}Mn into the aqueous phase thus indicating no association of Mn within a chlorophyll molecule. The residue from lipid extraction contained virtually all the manganese. This fraction undoubtedly is not homogeneous but because of the high percentage of nitrogen of the dry weight we have labeled it protein.

Additional evidence against the hypothesis of a manganese chlorophyll is shown in Fig. 10. Here it is shown that with increasing acetone (-15°)

concentration it is possible to extract all chlorophyll without extracting any appreciable amount of manganese. Thus although it seems clear that there is no manganno-chlorophyll, the possibility of a protein-Mn-chlorophyll complex is not excluded.

The amount of manganese per chlorophyll given by our data center around the number of 46 moles of chlorophyll per gram atom of manganese, a value somewhat of a mean of values previously reported in the literature. Curiously this ratio of Chl/Mn approximates the pool-size responsible for the O_2-gush[20] observed by many workers of ~ 1 O_2/150 Chl,[21,22] or ~ 1 Eq/40 Chl. This ratio of Chl/Mn is found regardless of chemical composition of buffer used in isolation of particles and more importantly independent of total phosphate concentration of particle. This latter observation is considered pertinent since mitochondria, chromatophores and chloroplasts are known to precipitate certain cations as their phosphates. This, of course, would lead to erroneously high values for the Chl/Mn ratio. Furthermore, if insoluble manganese phosphates constituted a sizable portion of the total chloroplast manganese-pool, one would expect that breakage of the chloroplast by sonication and subsequent centrifugations would separate the more dense insoluble phosphates from the less dense lipid-protein material. Our data do not indicate this, but do indicate that sonication alone is insufficient in breakage of the manganese bonding.

Manganese-binding in chloroplasts of Scenedesmus has been investigated with parameters such as pH, ionic strength, effect of detergents, protein denaturants and chelating agents. Such information was considered necessary for work towards isolation of the manganese carrier.

At pH values of 6.3-6.5 (Fig. 11) the manganese associated with the particles is not easily removed by washing or dialysis. On either side of this narrow pH range, however the binding of manganese is decreased considerably. The manganese released by adverse pH is not precipitable by $(NH_4)_2\ SO_4$ and is dialyzable thus indicating that the manganese released in this manner is not protein-bound. It is curious that the pH range for stability of manganese binding is similar to the pH range for stability of the Hill-reaction. We have not made, however, any measurements indicating a correlation of half-lives between the two.

At pH 6.5 chelating agents such as those shown in Table II do not effectively release the particle-bound manganese with the possible exception of KCN. In addition little exchange between the ^{54}Mn-particle bound manganese and the Mn^{2+} and MnO_4^{-1} is found, an observation consistent with the fact that we have not been able to exchange $^{54}Mn^{2+}$ into unlabeled chloroplasts in a wide variety of different conditions. These observations are, in general, consistent with those of Spencer and Possingham[6] and point to an extremely low dissociation value of chloroplast manganese. However, upon prolonged storage at 4° or -15° but not at -196° or with conditions common for protein denaturation, the chelating agents become increasingly more effective in removing the manganese.

In fact, simple incubation at neutral pH of particles with denaturants such as urea, thiourea, or guanidine results in a time, concentration, and temperature dependent release of manganese. The data of Fig. 12 show a $\sim$ 60% loss of bound manganese within 10 minutes by 6M urea treatment at 4°. At room temperature the release is more rapid and essentially complete.

High salt, such as with saturating concentrations of ammonium sulfate, do not result in release of manganese. Such results suggest a binding of manganese by protein which is released upon denaturation.

Although it is possible to solubilize the manganese protein in micellar form with Triton (Fig.13), digitonin and phosphatide micelles are ineffective. Micelles as prepared with Triton can be broken with $(NH_4)_2\ SO_4$ resulting in formation of lipo-protein layer and a clear hypophasic layer. The manganese is found associated with the lipo-protein layer unless again the micelles have been treated with urea, guanidine, heated 5 minutes at 50°, or other means generally used for protein denaturation. At -196° the micelles may be stored, but overnite storage at -15°, 4° and 23° results in extensive (70-100%) loss of the manganese to the hypophase upon ammonium sulfate treatment. Such behaviour points to extreme lability of the manganese-protein bond in the micelle.

By other procedures it has been possible to obtain a protein containing manganese into solution and demonstrate adsorption onto DEAE with subsequent elution at high ionic strengths. The great instability of the soluble protein and the lack of a specific "enzymatic" assay has hampered purification and critical assessment of such a protein(s) in its relationship to its function within photosystem II.

1. Pirson, A., Zeit. Bot. 31, 193 (1937).

2. Kessler, E., Arch. Biochem. Biophys. 59, 527 (1955).

3. Kessler, E., Arthur, W. and Brugger, J. E., Arch. Biochem. Biophys., 71, 326 (1957).

4. Eyster, C., Brown, T. E., Tanner, H. A. and Hood, S. L., Plant Physiol., 33, 235 (1958).

5. Kessler, E., Moraw, R., Rumberg, B. and Witt, H. T., Biochim. Biophys. Acta, 43, 134 (1960).

6. Spencer, D. and Possingham, J. V., Austral. J. Biol. Sci., 13, 441 (1960).

7. Spencer, D. and Possingham, J. V., Biochim. Biophys. Acta, 52, 379 (1961).

8. Lumry, R. and Spikes, J. D., In: "Research in Photosynthesis" (H. Gaffron, ed.) p. 373, Interscience, New York.

9. Bishop, N. I. and Gaffron, Hans., Biochem. Biophys. Res. Commun., 8, 471 (1962).

10. Kok, B., Rurainski, H. J. and Harmon, E. A., Plant Physiol., 39, 513 (1964).

11. Hoch, G. and Martin, I., Arch. Biochem. and Biophys., 102, 430 (1963).

12. Brody, S. S. and Brody, M., Arch. Biochem. Biophys., 95, 521 (1961).

13. Kok, B., in: "Photosynthetic Mechanisms of Green Plants." Nat. Acad Sci. Nat. Res. Council, No. 1145, p. 45 (1963) Washington, D.C.

14. Kerridge, D., J. Gen. Microbiology, 19, 497 (1958).

15. Siegler, M. R. and Sisler, H. D. Biochim. Biophys. Acta, 87, 70 (1964).

16. Siegler, M. R. and Sisler, H. D., Biochim. Biophys. Acta, 87, 83 (1964).

17. Kirk, J. T. O. and Allen, R. L., Biochem. Biophys. Res. Commun., 21, 523 (1965).

18. Arnon, D. I., In: "Trace Elements" (C. A. Lamb, O. G. Bentley and J. M. Beattie, Eds.) p. 1, (1957) Academic Press, New York.

19. Park, R. B. and Pon, N. G., J. Mol. Biol., 6, 105 (1963).

20. Blinks, L. R. and Skow, R. K. Proc. Natl. Acad. Sci., 24, 420 (1938).

21. deKouchkovsky, Y. and Briantais, J. M. In: "Photosynthetic Mechanisms of Green Plants."

22. Fork, D. C., Plant Physiol., 38, 323 (1963).

TABLE I

Distribution of ^{54}Mn in Fractions from Chloroplast Particles from ^{54}Mn-Uniformly Labeled Scenedesmus.

	Total Mn	Per Cent of Dry Weight	
	(cpm)	Chlorophyll	Nitrogen
Chloroplast Suspension	372,400	6.3	10.7
Protein Fraction	352,000	0.0	16.5
Lipid Fraction	890	22.0	1.1

TABLE II

EFFECT OF WASHING OF CHLOROPLAST PARTICLES

ON REMOVAL OF MANGANESE

Suspending Medium	Total Manganese in Particle (Original) (cpm)	(Final)	Percent of Total Manganese Removed
0.02M K Phos. pH 7.0	8984	7209	19.8
0.02M K Phos. pH 8.1	9127	6412	29.6
0.02M K Phos. pH 6.5	9230	8660	6.5
0.02M + $1x10^{-3}$M EDTA	9301	8596	8.0
0.2M + $1x10^{-4}$M Hydroxyquinoline	9285	8913	4.0
0.02M + $1x10^{-4}$M O-phenanthroline	9369	8669	7.5
0.02M + $1x10^{-3}$M KCN	9185	7451	18.8
0.02M + $1x10^{-4}$M Diethyldithiocarbamate	9232	8639	6.5
0.02M + $1x10^{-4}$M $MnCl_2$	9334	8111	13.0
0.02M + $1x10^{-5}$M $KMnO_4$	9286	8656	6.6

Fig. 1 Effect of moderate-level of Mn deficiency on photosynthesis. Photosynthesis measurements were made with a Clark-type O_2 electrode using 0.5 mil Teflon Type A membrane. Scenedesmus D_3 cells (48 hour culture) containing 20 μgrams $Chlorophyll_{total}$ were illuminated at 25° in 0.1M Na_2CO_3-0.1M $NaHCO_3$, pH 9.4, saturated with 5% CO_2-Air. Total volume of vessel was 1.13 ml. Irradiation was of wavelength > 500 mμ. Full intensity corresponded to 4000 ft. candles. Values are corrected for dark respiration.

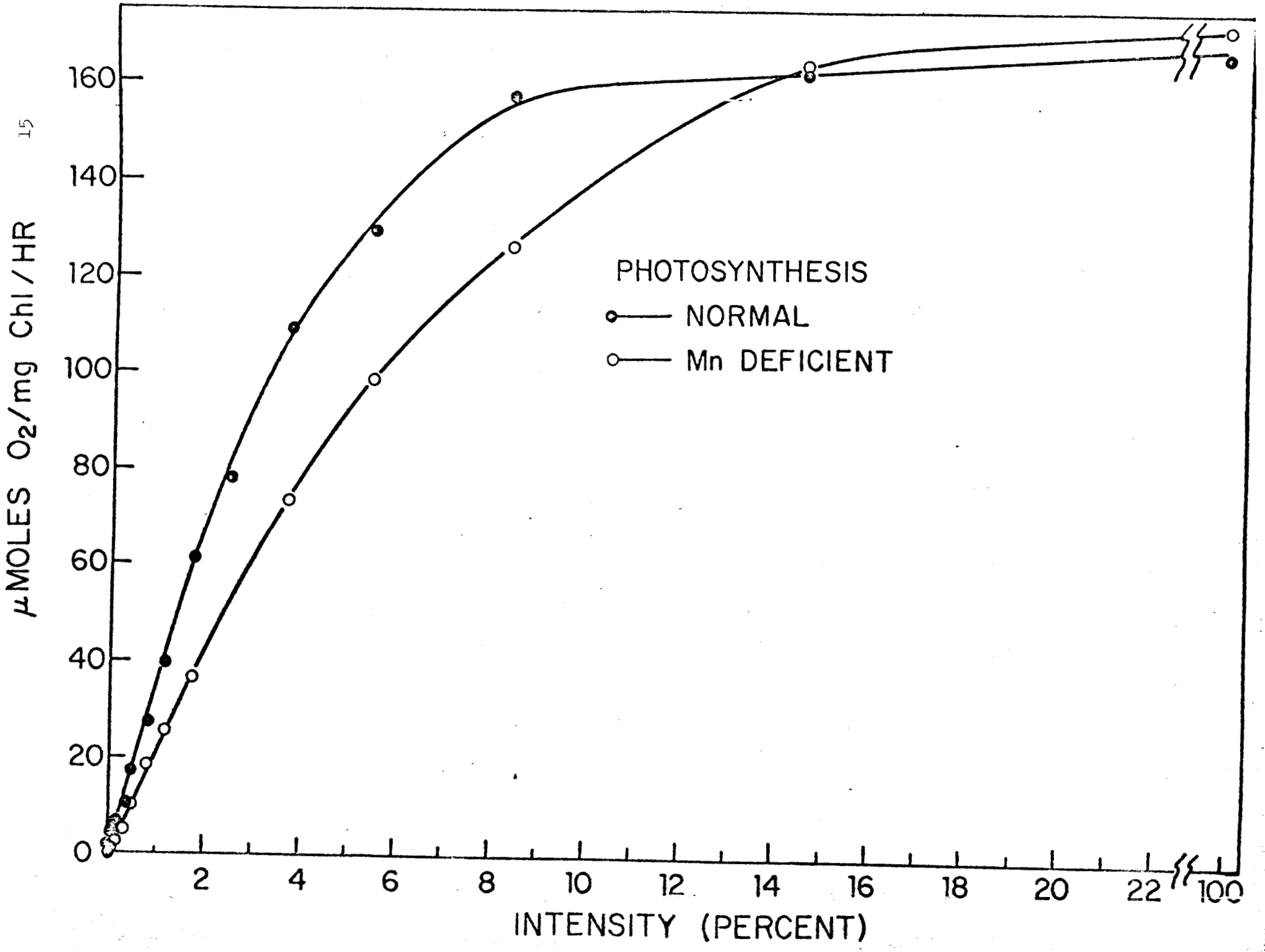

160
140
120
100
80
60
40
20
0
μMOLES O2/mg Chl/HR
PHOTOSYNTHESIS
NORMAL
Mn DEFICIENT
2
4
6
8
10
12
14
16
18
20
22
100
INTENSITY (PERCENT)

Fig. 2 Effect of extreme level of Mn deficiency on photosynthesis and quinone-Hill reaction. Conditions were the same as described in legend of Fig. 1 except that extremely Mn-deficient cells (48 hour culture) were used where noted. Quinone-Hill reaction was run at pH 6.5 using freshly sublimed benzoquinone.

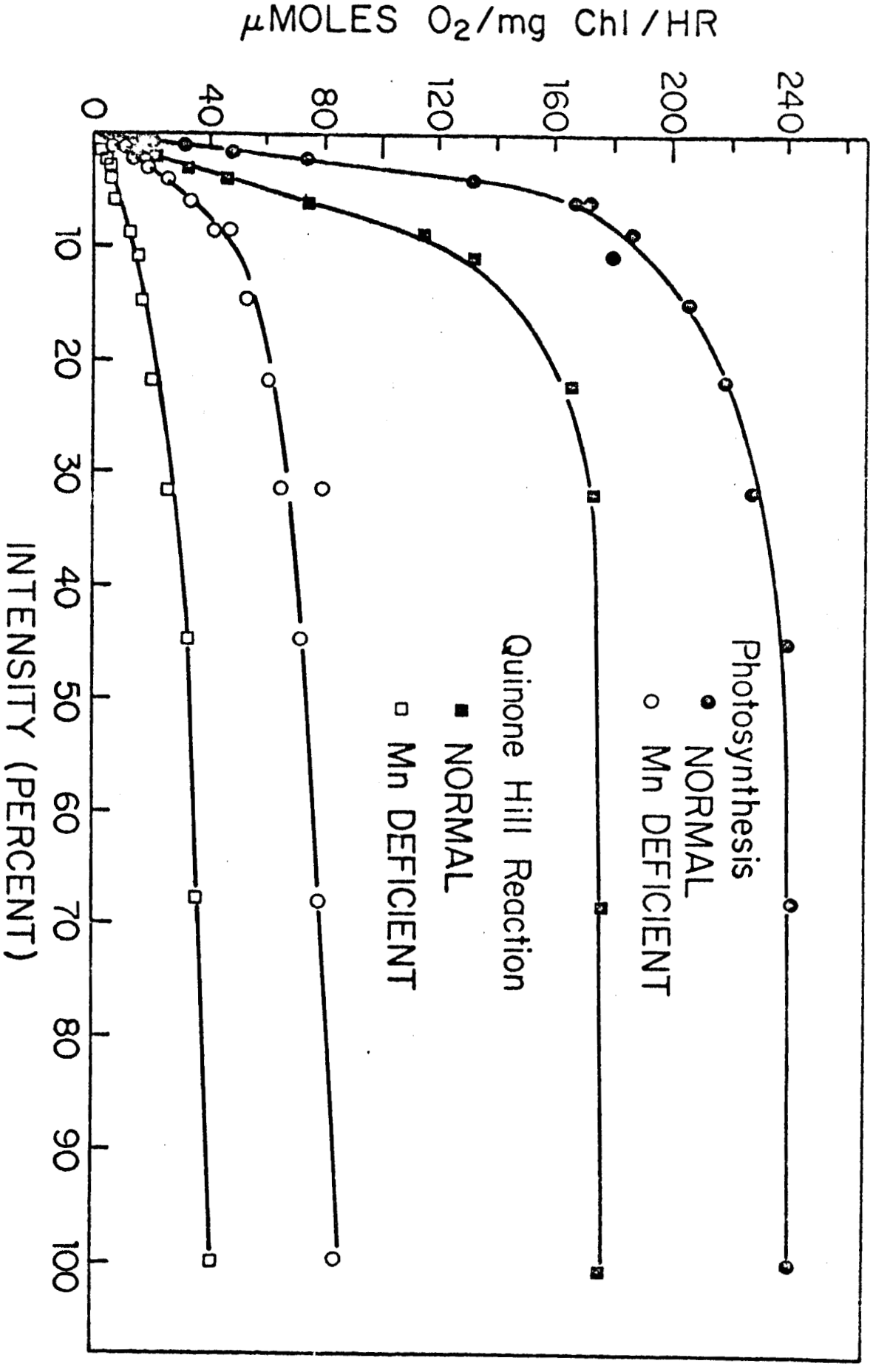

μMOLES O2/mg Chl/HR
0
40
80
120
160
200
240
10
20
30
40
50
60
70
80
90
100
INTENSITY (PERCENT)
Photosynthesis
● NORMAL
○ Mn DEFICIENT
Quinone Hill Reaction
■ NORMAL
□ Mn DEFICIENT

Fig. 3 Hill-reaction activity of chloroplast particles from cells used in experiments of Fig. 2. Reaction vessel contained the following in micromoles: Tris-HCl, pH 7.2, 50; NaCl, 10; and FeCN, 1, in total volume of 1.13 ml containing 20 μgrams $Chlorophyll_{total}$. For other details see legend of Fig. 1.

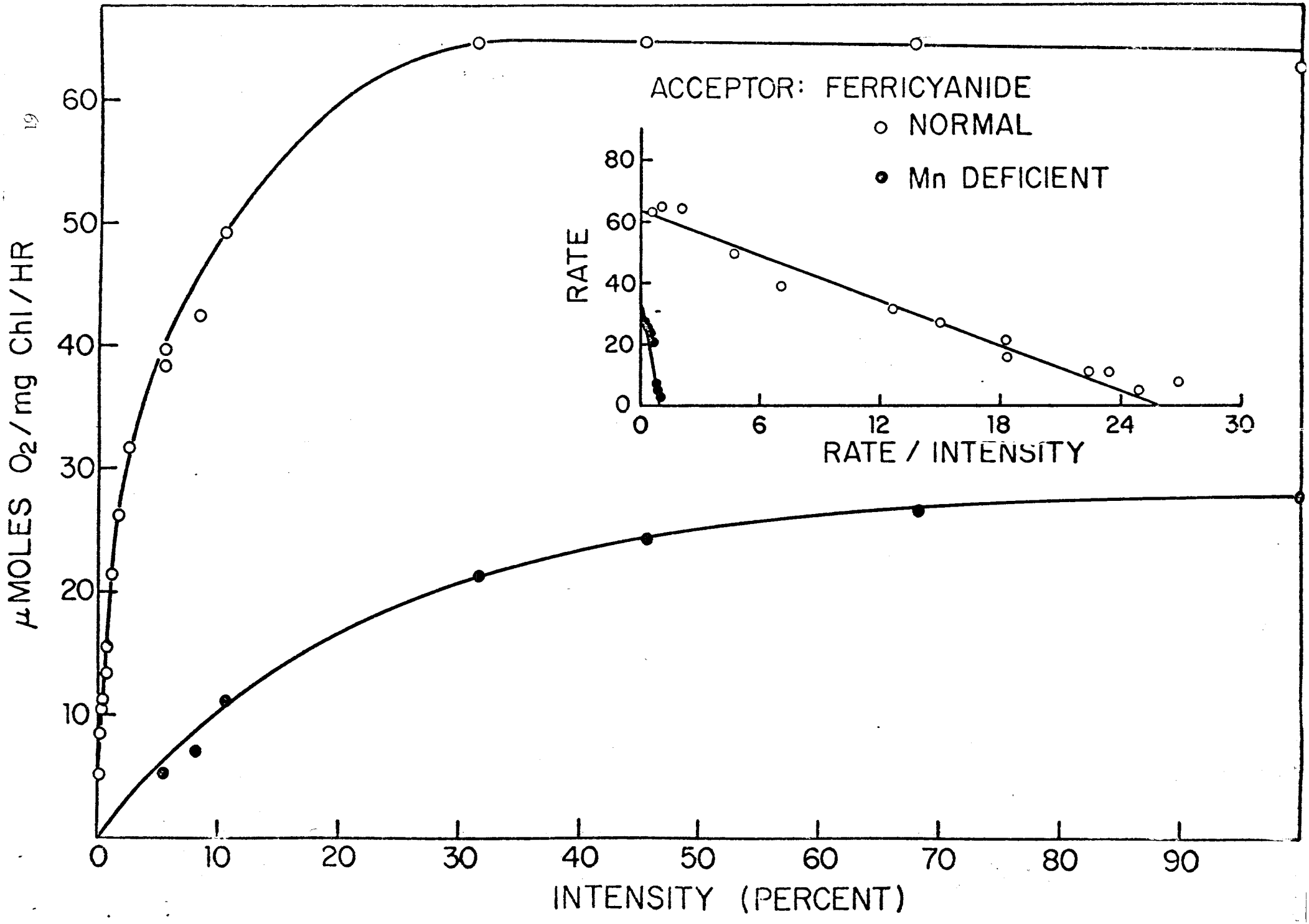

ACCEPTOR: FERRICYANIDE
NORMAL
Mn DEFICIENT
RATE
RATE / INTENSITY
μMOLES O2 / mg Chl / HR
INTENSITY (PERCENT)
0
10
20
30
40
50
60
70
80
90
6
12
18
24

Fig. 4 Reduced cytochrome c photo-oxidation with digitonin treated particles from normal and extremely deficient algae used in experiments of Fig. 2. The reaction mixture contained in micromoles: K Phos buffer, pH 6.8, 10; 0.15M $MgCl_2$ 7.5; NaCl, 17.5; methylviologen, 0.1; KCN, 0.2; cytochrome c (92% reduced) 1.0 mg; plastocyanin, 0.005; and 20 μgrams $Chlorophyll_{total}$ in 1.13 ml. For other details see legend of Fig. 1.

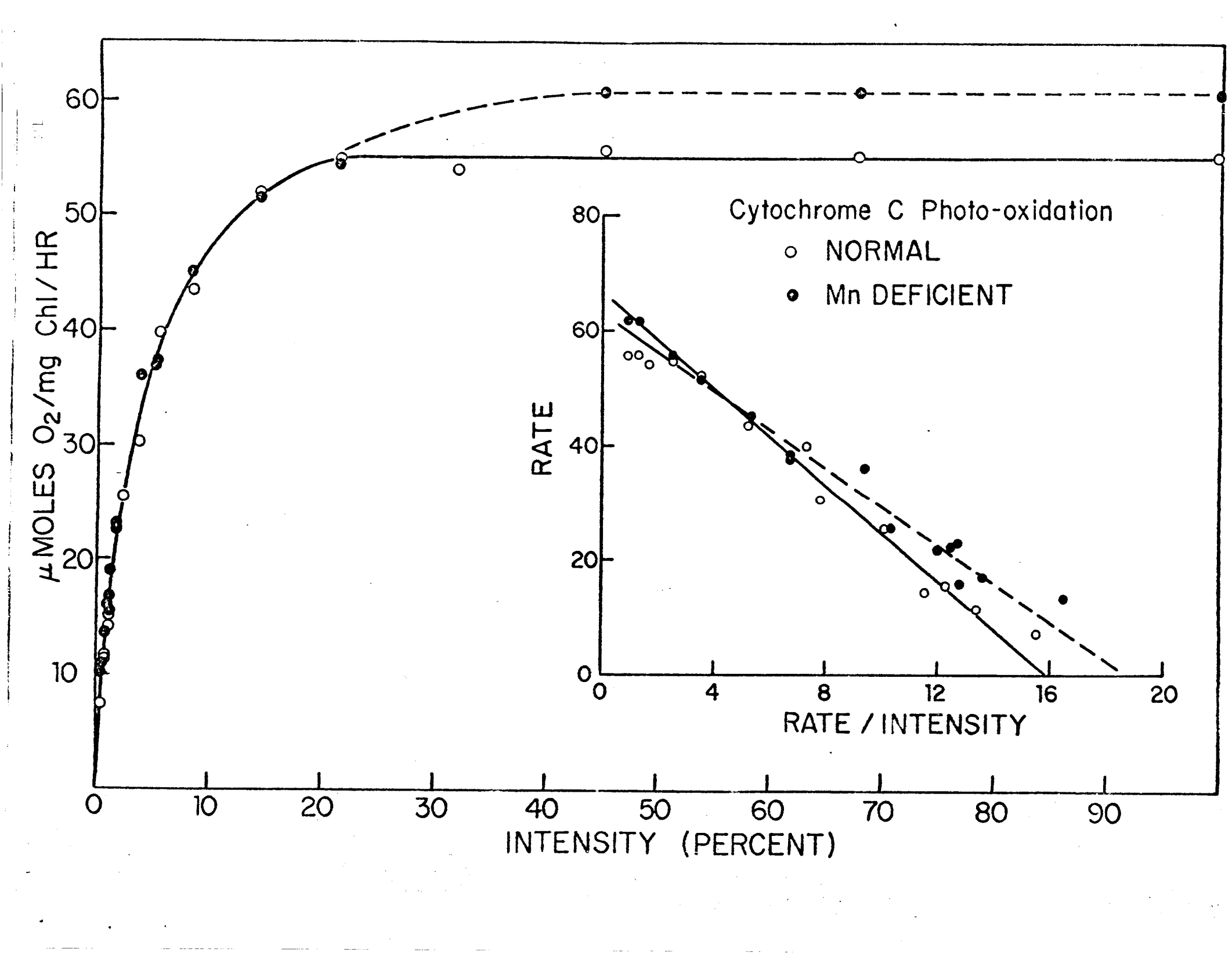
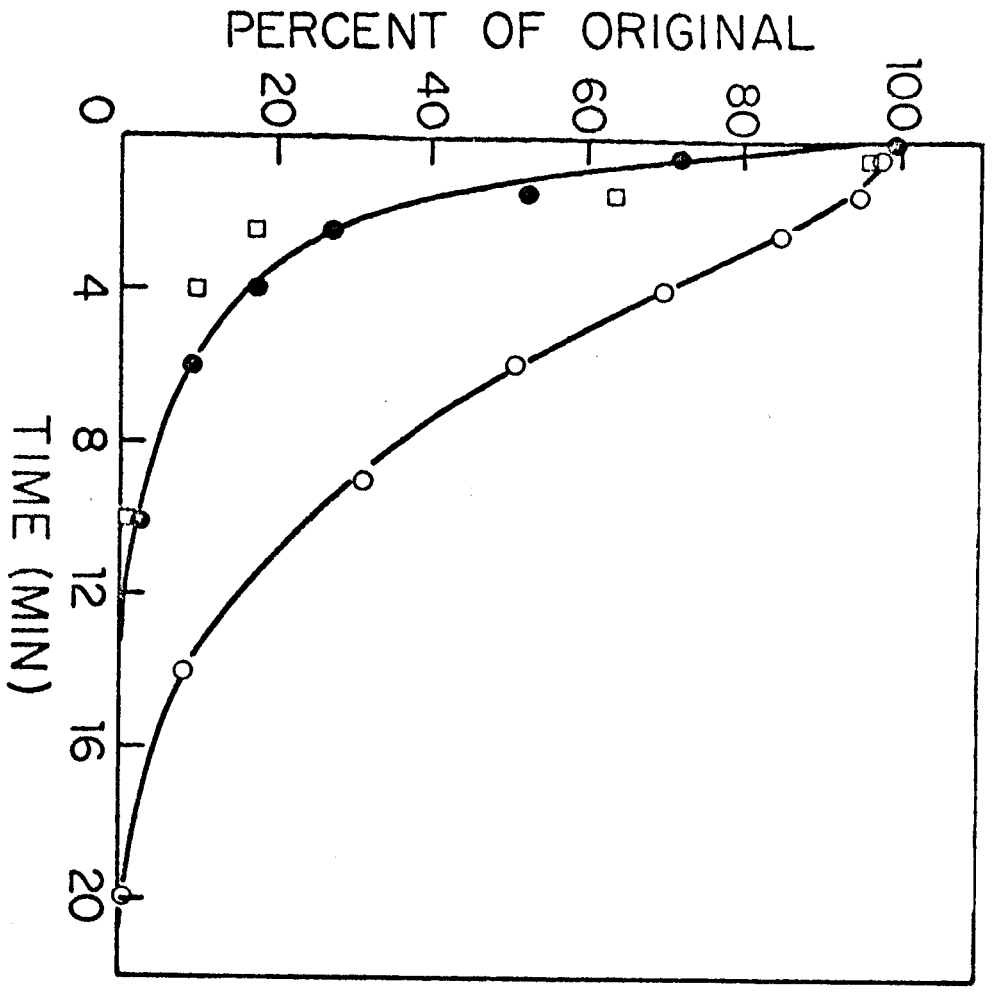

60
50
40
30
20
10
μMOLES O2/mg Chl/HR
0
10
20
30
40
50
60
70
80
90
INTENSITY (PERCENT)
Cytochrome C Photo-oxidation
NORMAL
Mn DEFICIENT
80
60
40
20
0
RATE
0
4
8
12
16
20
RATE / INTENSITY

Fig. 5 Effect of treatment at 50° on loss of Hill-activity and ^{54}Mn-labeled particles from ^{54}Mn-uniformly labeled Scenedesmus. See legend of Fig. 3 for details. At indicated time, aliquots of particles were added to .02M Phos pH 6.7 containing 0.001M versene at 4° and particles removed by centrifugation. ^{54}Mn was determined as described in Methods.


PERCENT OF ORIGINAL
0
20
40
60
80
100
TIME (MIN)
0
4
8
12
16
20

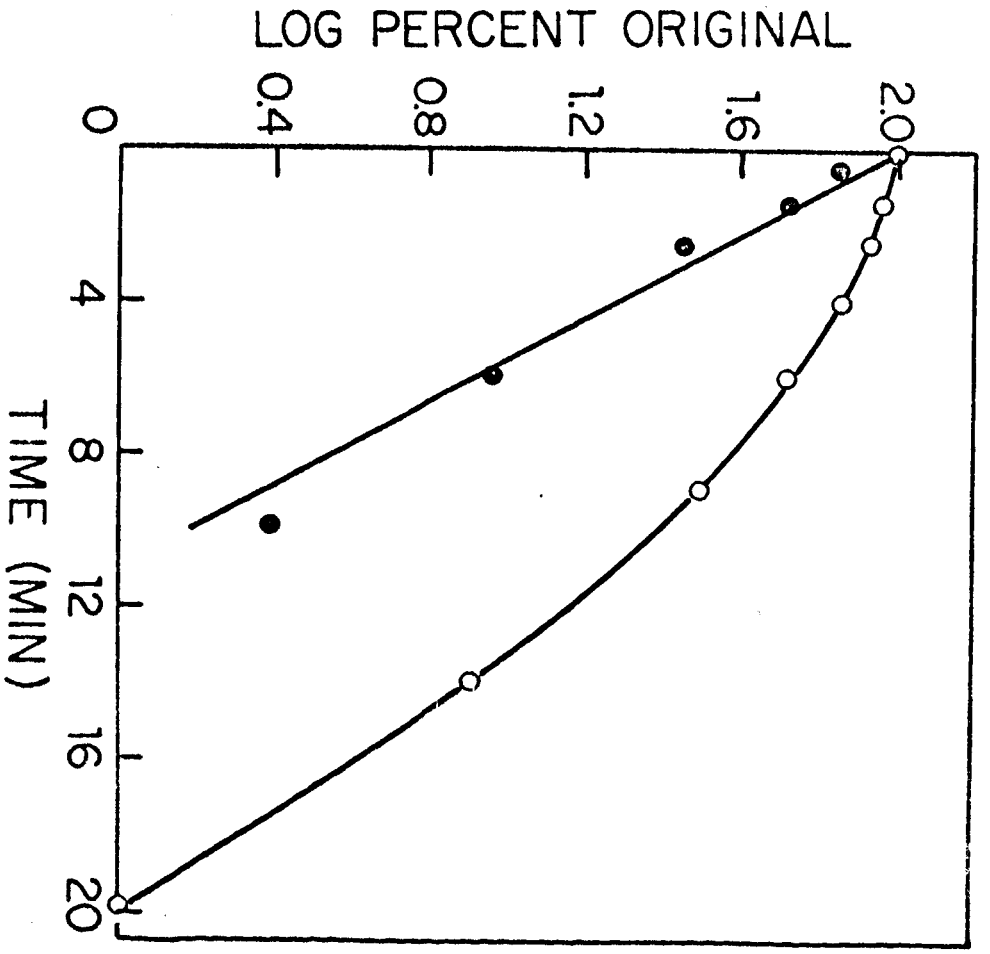

LOG PERCENT ORIGINAL
0
0.4
0.8
1.2
1.6
2.0
TIME (MIN)
0
4
8
12
16
20

Fig. 6 Difference spectrum of 80% acetone extraction of particles from normal and extremely Mn deficient Scenedesmus. Absorbance at 663 mμ in both sample (normal) and reference (deficient) cuvette equal to 0.615. Trace recorded on Cary Model 15.

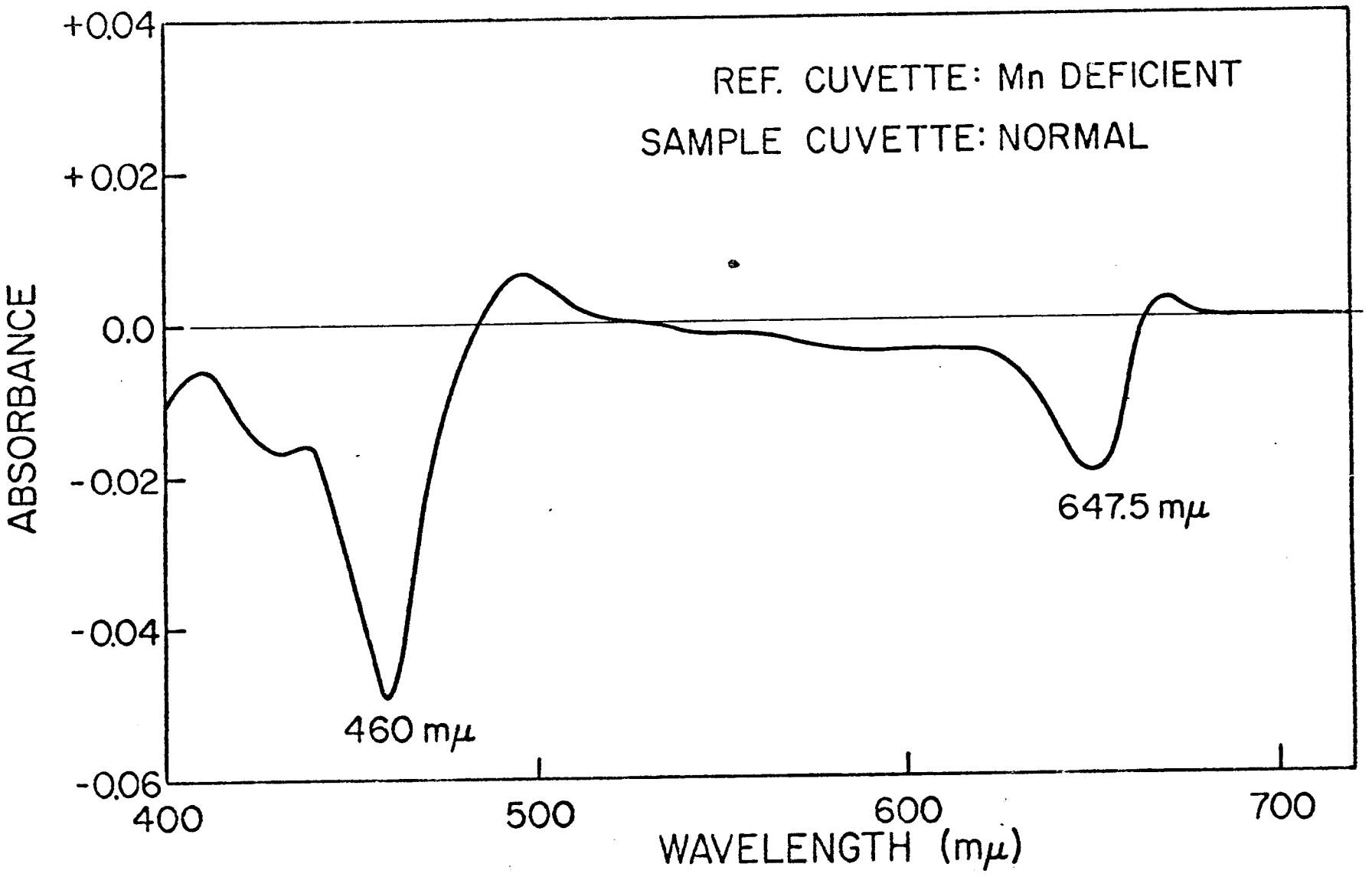

REF. CUVETTE: Mn DEFICIENT
SAMPLE CUVETTE: NORMAL
+0.04
+0.02
0.0
-0.02
-0.04
-0.06
ABSORBANCE
460 mμ
647.5 mμ
400
500
600
700
WAVELENGTH (mμ)

Fig. 7 Relative fluorescence emission at -196° of normal and extremely Mn-deficient Scenedesmus D_3. Samples had equal amounts of $chlorophyll_{total}$. Particles from such cells showed essentially the same fluorescence emission.

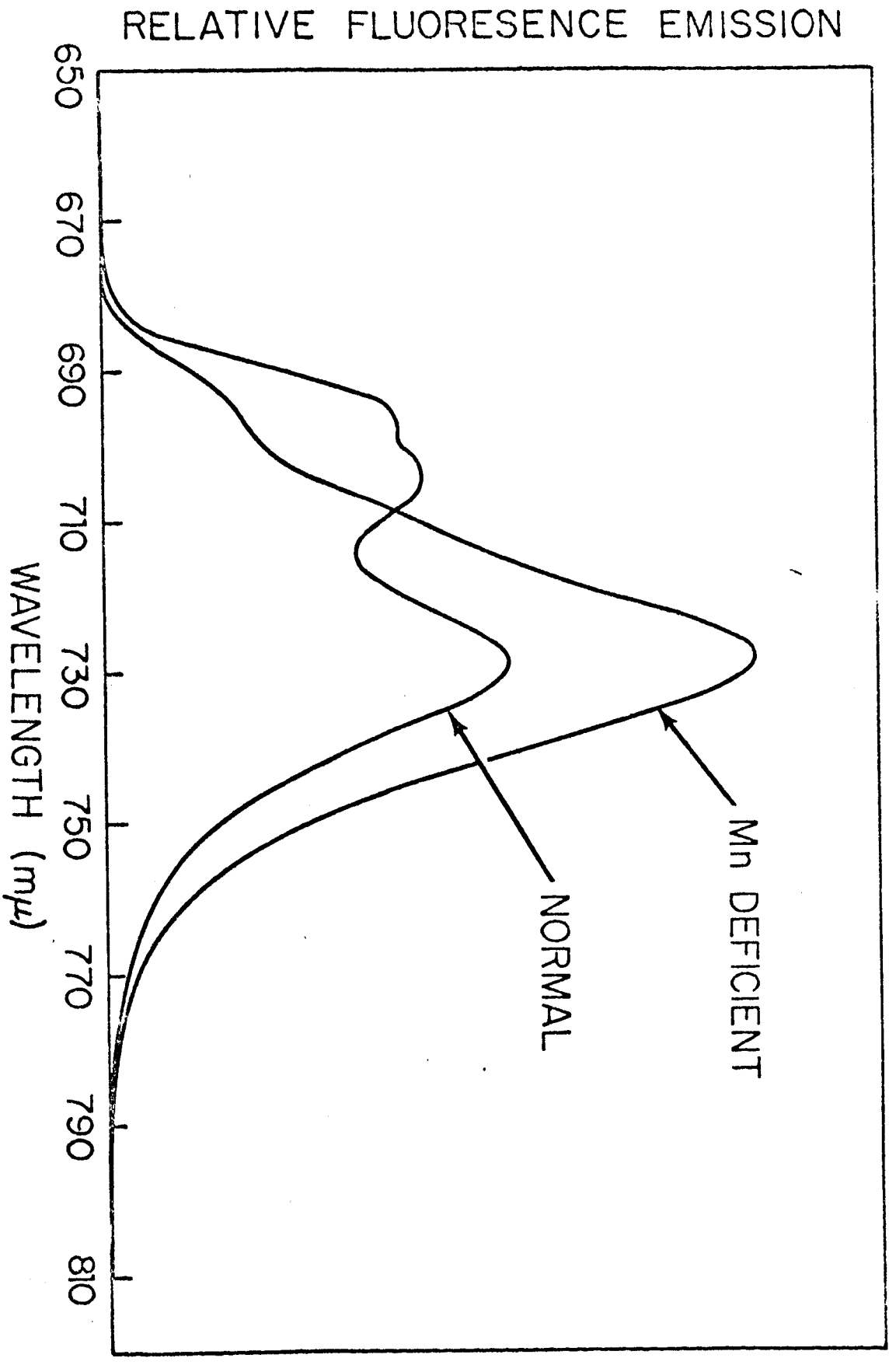

RELATIVE FLUORESCENCE EMISSION
650
670
690
710
730
750
770
790
810
WAVELENGTH (mμ)
NORMAL
Mn DEFICIENT

Fig. 8 Course of chlorophyll synthesis during reactivation of manganese deficient Scenedesmus. Open triangles - illuminated, non-deficient. Closed triangles - illuminated non-deficient cells with cycloheximide (5 µg/ml) added. Open squares - illuminated deficient cells with cycloheximide (5 µg/ml) and Mn^{2+} added; or deficient cells in darkness with Mn^{2+}. Open circles - illuminated deficient cells with Mn^{2+} added.

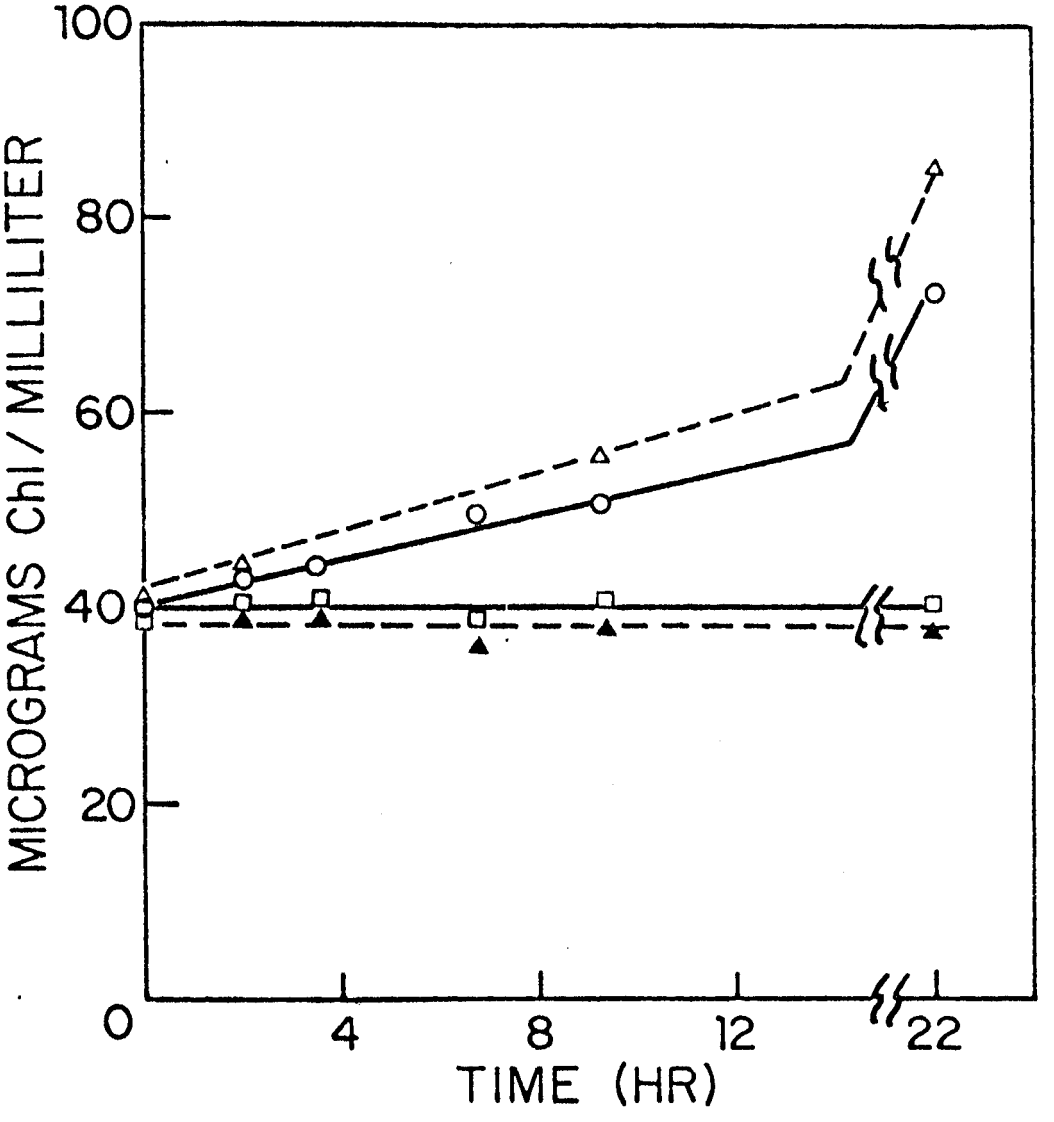

100
80
60
40
20
0
MICROGRAMS Chl / MILLILITER
4
8
12
22
TIME (HR)

Fig. 9 Reactivation of quinone-Hill reaction by light and Mn^{2+} in absence of net chlorophyll and protein synthesis. Closed circles - deficient cells illuminated in absence of Mn^{2+}, or with Mn^{2+} and without illumination. Open circles - deficient cells illuminated, Mn^{2+} added. Open squares - deficient cells illuminated, Mn^{2+} and cycloheximide (5 μg/ml) added. Closed triangles - non-deficient cells; Open triangles - non-deficient cells with cycloheximide. Cells were suspended in growth medium containing all trace elements including Co^{2+} and VO_3^{-1}. At zero time Mn^{2+} ("Spec-pure", 0.5×10^{-5}M) was added. At indicated times, aliquots were removed and assayed for quinone-Hill reaction, total chlorophyll, and incorporation of ^{14}C-phenylalanine into TCA-precipitable protein. Protein synthesis was inhibited ~ 90% by cycloheximide throughout course of experiment.

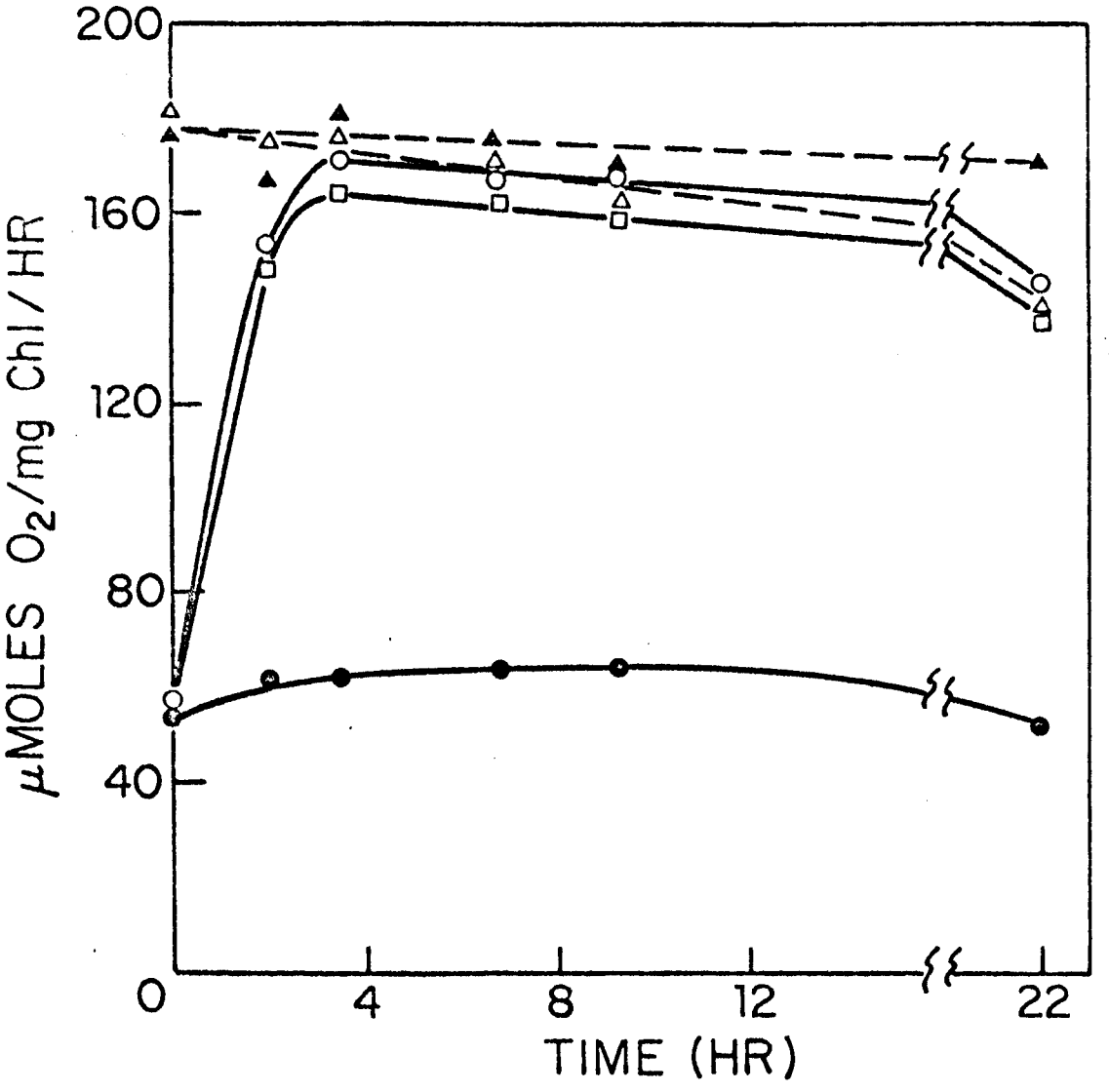

200
160
120
80
40
0
4
8
12
22
μMOLES O2/mg Chl / HR
TIME (HR)

Fig. 10 Effect of acetone on extraction of chlorophyll and ^{54}Mn from particles obtained from ^{54}Mn-uniformly labeled Scenedesmus. Closed circles - Chlorophyll; Closed squares - ^{54}Mn in acetone extractions; Open circles - ^{54}Mn extractable by buffer following acetone extraction.

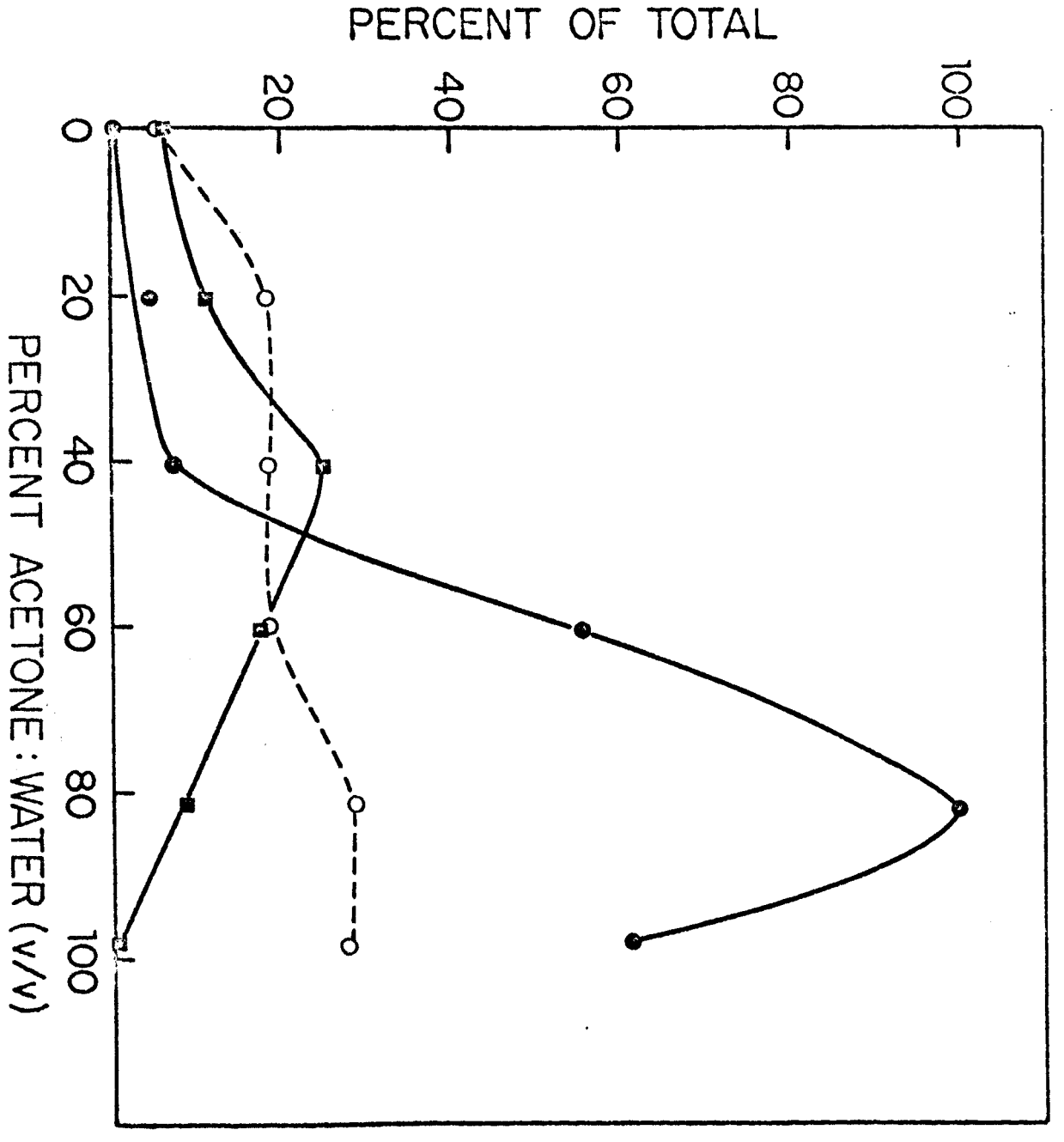

PERCENT OF TOTAL
0
20
40
60
80
100
PERCENT ACETONE : WATER (v/v)
0
20
40
60
80
100

Fig. 11 Effect of pH on loss of chloroplast bound manganese. Particles incubated at 5° for 21 hours in 0.02M buffers then removed by centrifugation. A total of 2200 cpm was used in each.

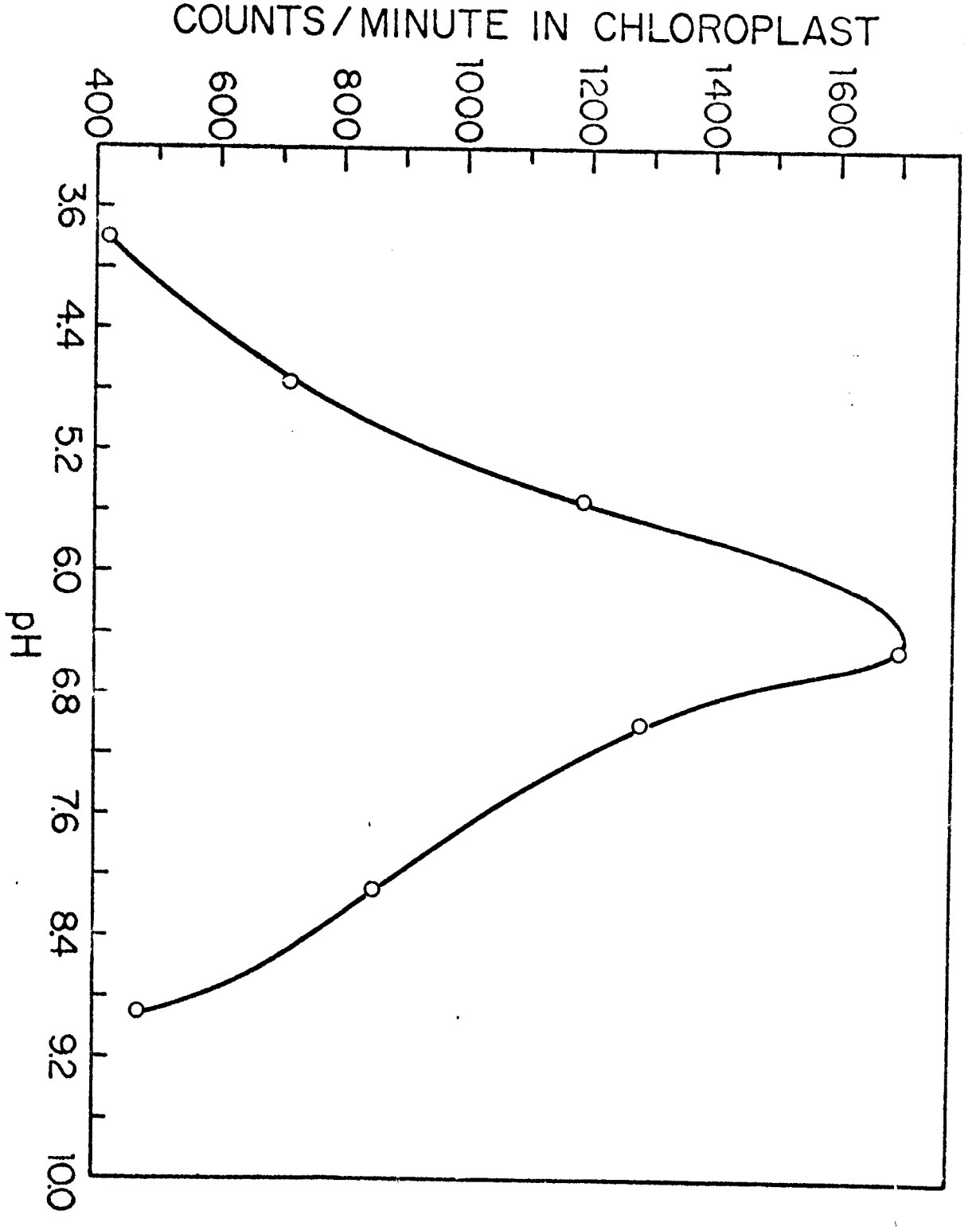

COUNTS / MINUTE IN CHLOROPLAST
400
600
800
1000
1200
1400
1600
pH
3.6
4.4
5.2
6.0
6.8
7.6
8.4
9.2
10.0

Fig. 12 Effect of urea concentration on loss of particle bound manganese. Labeled particles were incubated for 10 minutes at 4^{o} in 0.02M K Phos pH 6.7 containing urea at concentrations specified then centrifuged to remove the particles.

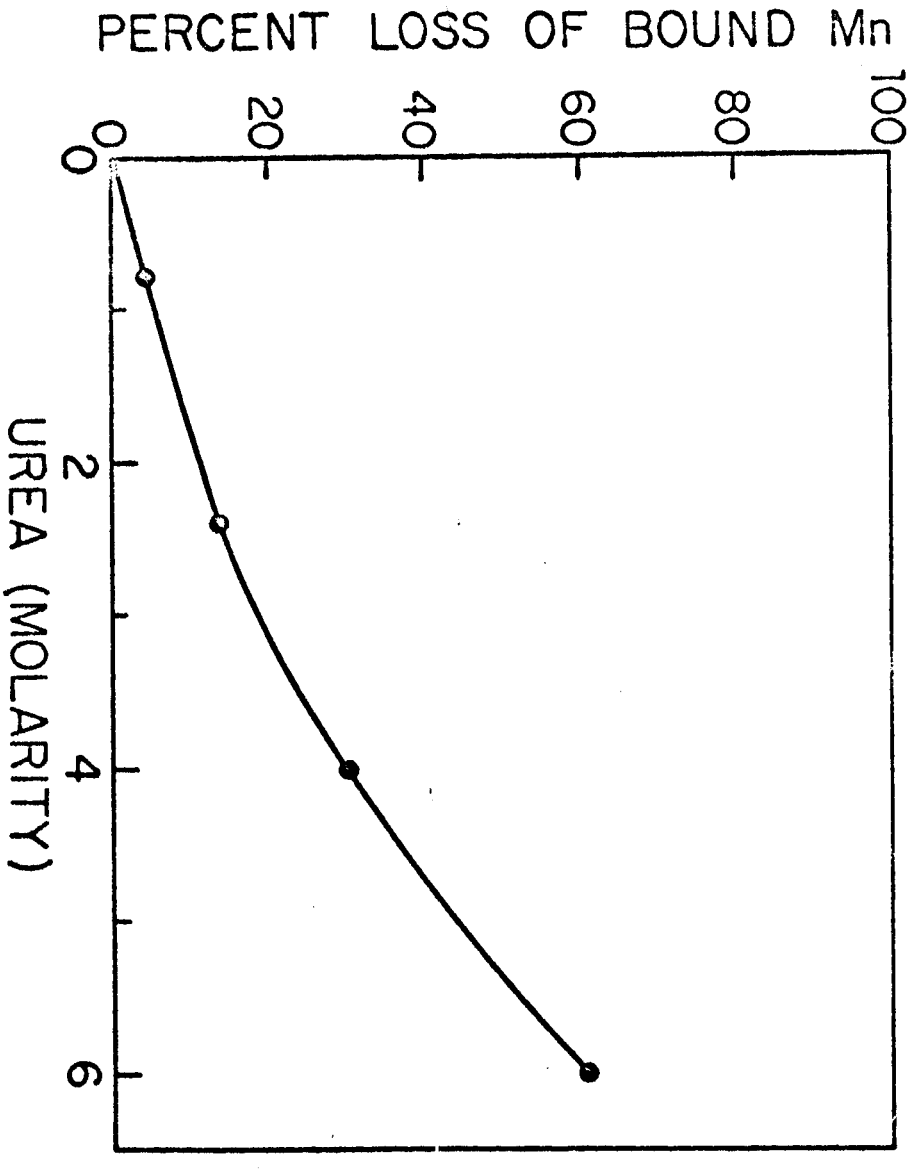
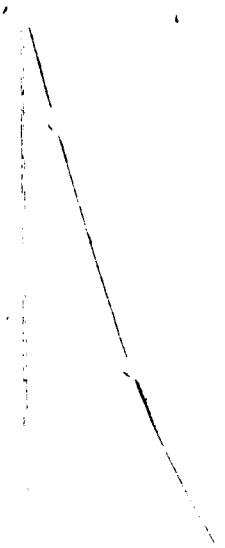
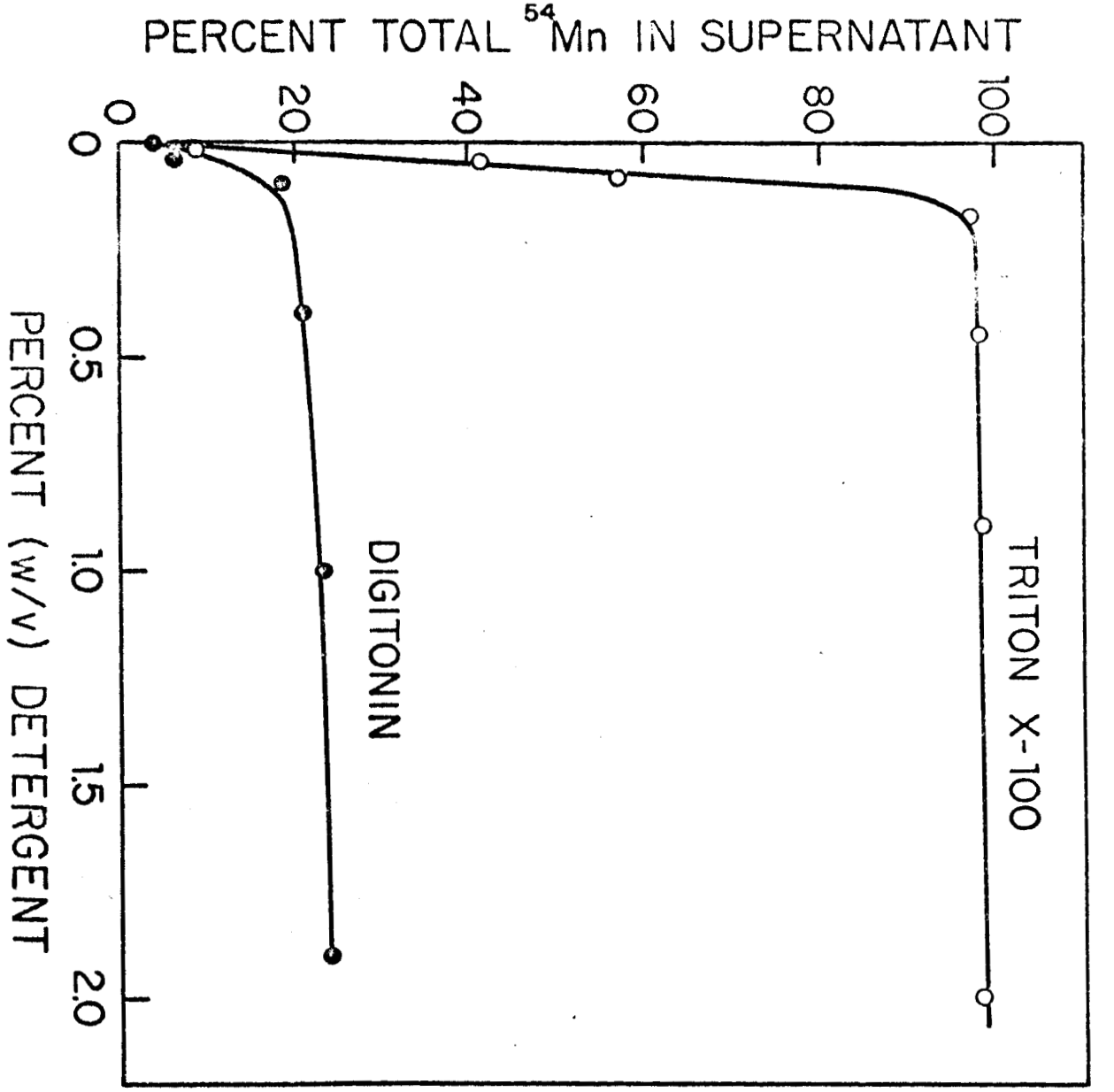

PERCENT LOSS OF BOUND Mn
0
20
40
60
80
100
0
2
4
6
UREA (MOLARITY)

Fig. 13 Detergent treatment of ^{54}Mn-labeled particles. Particles (100 μgrams Chl) were incubated at 4° for 1 hour with the indicated concentration of detergent then centrifuged at 144,000 x g for 1 hour.




PERCENT TOTAL ^{54}Mn IN SUPERNATANT
0
20
40
60
80
100
0
0.5
1.0
1.5
2.0
PERCENT (w/v) DETERGENT
DIGITONIN
TRITON X-100